

Instructions for Use of E.coli Residual DNA Detection Kit (qPCR)

The kit is intended for scientific research only and should not be used for diagnosis

Cat. No. HG- ED001

Introduction

The E.coli Residual DNA Detection Kit (qPCR) is designed for the quantitative detection of E.coli host cell DNA in intermediates, semi-finished products and finished products of various biological products. This kit adopts the principle of Taqman probe to quantitatively detect E.coli residual DNA in samples. The kit is a rapid, specific and reliable device, with the minimum detection limit reaching fg level.

Specification

100 Reactions

Kit components

Components	Specification	Preparation
<i>E. coli</i> DNA quantitative standard	50 μ L \times 1 vial	-18 $^{\circ}$ C and below
<i>E. coli</i> Primer&Probe MIX	550 μ L \times 1 vial	-18 $^{\circ}$ C and below, protected from light
2x qPCR Reaction Buffer	1.1 mL \times 1 vial	-18 $^{\circ}$ C and below, protected from light
DNA diluent	1.0 mL \times 3 vials	-18 $^{\circ}$ C and below
ROX High*	50 μ L \times 1 vial	-18 $^{\circ}$ C and below, protected from light
ROX Low*	50 μ L \times 1 vial	-18 $^{\circ}$ C and below, protected from light

* Please select appropriate ROX for corresponding model.

Table 1: Kit components

Storage conditions and shelf life

The shelf life is 18 months when stored at -18 $^{\circ}$ C and below.

Applicable models

Including but not limited to

- ◆ ABI PRISM 7500
- ◆ CFX96(Bio-Rad)
- ◆ Bioer Linegene 9600plus

When using with different instrument models, please select the appropriate reference stain ROX.

Instruments	ROX reference stain
Applied Biosystems® 5700, 7000, 7300, 7700, 7900HT, StepOne™, and StepOnePlus™	ROX High
Applied Biosystems® 7500, ViiA™ 7, QuantStudio™ 12K Flex, Agilent Mx3000P™, Mx3005P™, and Mx4000™	ROX Low
Rotor-Gene™, DNA Engine Opticon™, Opticon™2, Chromo 4™ Real-Time Detector, Mastercycler®ep realplex, Smart Cycler®, Roche LightCycle@480, Roche LightCycler@Nano, Bio-Rad CFX96, and Illumina Eco™	No ROX

Prepared by the user

- ◆ 1.5 mL low-attachment sterile centrifuge tube
- ◆ qPCR plate or strip tubes

Operation procedures

I. Dilution of DNA quantitative standard and preparation of standard curve

The concentration of *E. coli* DNA quantitative standard is 30 ng/μL, as indicated on the vial wall label.

1. Take out the DNA quantitative standard and DNA diluent, place them on ice to thaw completely, then gently shake to mix well, and perform instantaneous centrifugation.
2. Take 6 clean 1.5 mL centrifuge tubes and label them as STD0, STD1, STD2, STD3, STD4, and STD5, respectively.
3. In the STD0 tube, dilute the DNA quantitative standard with DNA diluent by 10 folds as STD0, shake to mix well, and perform rapid centrifugation for 10 seconds.
4. Add 90 μL of DNA diluent to STD1, STD2, STD3, STD4, and STD5 tubes, respectively.
5. The dilution of standards is present in the following table:

Standard No.	Dilution volume	Concentration (fg/μL)
ST1	10 μL STD0 + 90 μL DNA diluent	3.00×10 ⁵
ST2	10 μL STD1 + 90 μL DNA diluent	3.00×10 ⁴
ST3	10 μL STD2 + 90 μL DNA diluent	3.00×10 ³
ST4	10 μL STD3 + 90 μL DNA diluent	3.00×10 ²
ST5	10 μL STD4 + 90 μL DNA diluent	3.00×10 ¹

Table 2. Dilution of standards

II. Preparation and addition of qPCR reaction solution

1. Calculate the required number of reaction wells based on the standard curve and the number of samples to be tested. Generally, 3 replicate wells are required for each sample.

Number of reaction wells = (5 concentration gradients on the standard curve + 1 no template control (NTC) + number of test samples) × 3.

- Calculate the total volume of qPCR MIX required for this analysis based on the number of reaction wells.

Volume of qPCR MIX = (number of reaction wells + 2) × 15 μL (including volume loss of 2 wells).

- Take out each reagent and place on ice to thaw, gently shake to mix well, and prepare qPCR MIX according to the table below:

Components	Reaction in single-well
2x qPCR Reaction Buffer	10μl
<i>E.coli</i> Primer&Probe MIX	4.6μl
ROX*	0.4μl
Total volume	15μl

* Please select appropriate ROX for corresponding model. If there is no ROX suitable for the model, please add deionized water (free of nucleic acid and nuclease contamination) of same volume

The loading to each reaction well is as follows:

Standard curve	15μl qPCR MIX +5μlSTD1/STD2/STD3/STD4/STD5
No template control (NTC)	15 μL qPCR MIX + 5 μL DNA diluent
Test sample	15 μL qPCR MIX + 5 μL test sample

The total liquid volume in each well should be 20 μL after loading. Gently shake the 96-well plate to mix well, perform rapid centrifugation for 10 seconds, and place the plate in the qPCR system.

III. qPCR program parameter setting

Taking the CFX96 qPCR system (BIO-RAD) as an example.

- Create an experimental reaction program and set up a two-step reaction program. The PCR reaction program is as follows: Pre-denaturation at 95°C for 10 minutes, at 95°C for 15 seconds, and at 60°C for 1 minute; repeat for 40 cycles, with the reaction volume of 20 μL.
- Create an experimental reaction plate, click on "Select Fluorophores" and select fluorescent FAM.

Select sample wells in the reaction plate chart, select "Unknown" in the "Sample Type" dropdown menu, check fluorescent FAM, and name the Target Name as "-DNA", then input the number of replicate wells and Sample Name for each sample.

Select standard curve wells in the reaction plate chart, select "Standard" in the "Sample Type" dropdown menu, check the fluorescence FAM, and name the Target Name as "-DNA", then input the number of replicate wells and Sample Name for each dilution gradient. Assign values of 300000, 30000, 3000, 300, and 30 (in fg/μL), respectively, to the "Concentration" column of STD1, STD2, STD3, STD4, and STD5.

- Click "Start Run" and choose the save path

IV. qPCR result analysis

Taking the CFX96 qPCR system (BIO-RAD) as an example.

1. Click "Quantitation" on the data analysis window to read the slope, intercept, amplification effect, and R^2 of the standard curve.
2. In the "Quantitation Data" window, the detection values (in fg/ μ L) of NTC and test samples can be read in the "SQ Mean" column.
3. For NTC, the result should be N/A, or the Ct value should be greater than the mean Ct value of the lowest concentration on standard curve.

Disclaimer

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EAGLE BIOSCIENCES, INC.

20A NW Blvd, Suite 112 Nashua, NH 03063

Phone: 617-419-2019 FAX: 617-419-1110

www.EagleBio.com info@eaglebio.com



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